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TENTACULAR NEMATOCYTE DISCHARGE AND "SELF-RECOGNITION" IN *ANTHOPLEURA ELEGANTISSIMA* BRANDT

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Abstract

In the clonal anemone *Anthopleura elegantissima* isolated mucus from both clonemates and non-clonemates fails to elicit discharge of tentacular nematocytes. While direct contact between clonemates fails to elicit significant discharge, similar contact between non-clonemates does so.

Isolated mucus from a number of xenogeneic Zoantharia elicits discharge, and the reciprocal tests are positive. However, while mucus from the congeneric *A. xanthogrammica* fails to elicit discharge in *A. elegantissima*, the latter's mucus effects significant discharge in *A. xanthogrammica.*

The relation of these and other studies to our understanding of the physiology of nematocyte discharge is discussed.

Introduction

The anemone *Anthopleura elegantissima* is a common inhabitant of the intertidal zone on the West Coast of North America. It occurs in two forms, one solitary and one aggregating (Hand, 1955; Francis, 1979). Francis (1973a) showed that assemblages of the latter consist of clonal animals produced by longitudinal fission. She further (Francis, 1973b) showed that tentacular contact between individuals of different clones (non-clonemates) results in rapid withdrawal of those tentacles involved, often of nearby tentacles and even of the whole tentacular crown. Repeated contact between non-clonemates eventually elicits elaborate agonistic behavior, producing anemone-free zones between competing clones. In contrast, individuals of the same clone associate closely with each other in dense aggregations, and clonemate tentacles interlace without apparent interaction.

These anemones possess a mucus coat. One may ask whether this mucus is involved in distinguishing self from non-self. Lubbock (1979) investigated the mucus antigenicity of a number of anemone species and found no difference within species, but marked difference between species. He suggested that anemone mucus may be species-specific and that it may be one factor used in recognition. We have used the level of tentacular nematocyte discharge as the response criterion to investigate the role of mucus in self-recognition.

Materials and Methods

All experiments were conducted in the Marine Laboratory of the University of California, Santa Barbara. Experimental animals consisted of the aggregating form of *A. elegantissima* from each of two phenotypically distinct clones, the solitary form of the same species, the congeneric *A. xanthogrammica*, and *Metri-*

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dium senile, Tealia lofotensis and *Corynactis californica.* All animals were kept in separate glass bowls in filtered, running seawater at 12°–13°C, under the normal day–night cycle of the laboratory (approx. 12 on and 12 off). The holding area and bowls were drained and rinsed frequently during the course of the experiments. All animals were acclimated to laboratory conditions for two weeks before the experiments began. At intervals they were fed small pieces of *Mytilus,* but never within 24 hours of experimentation.

An anemone whose responses were to be tested was held in a glass bowl supplied with oxygenated seawater. The bowl was placed under a dissecting microscope for observation. To avoid heat stress lighting was kept at the absolute minimum required for clear observation. Only anemones with attached pedal disks and expanded crowns were used. All experiments were conducted between 1200 and 1400.

The standard test for discharge was a modification of the technique of Davenport *et al.* (1961). A glass rod with adhered mucus was brought in contact with the tip of an arbitrarily chosen tentacle of the subject animal. If, upon moving the rod away, rod and tentacle adhered, the test was scored a positive; if not, a negative. Adhesion was assumed to result from nematocyte discharge. With this technique, variation of the intensity of contact is rendered insignificant *only* by large sample size. Accordingly, N = 100 in all test series. The rods used in the test were lightly etched with 48% hydrofluoric acid for better adhesion of mucus. Before use they were sterilized in 95% ethanol, flamed over a Bunsen burner and cooled. Occasionally after flaming residual carbon on a rod may elicit nematocyte discharge. As a control for this a rod to be used in a test was first brought in contact with a tentacle of the anemone about to be used as a donor of mucus. If the rod adhered, it was discarded. If it did not, it was then brought in contact with the oral disk or upper column of the donor until mucus had adhered to it. Test of the subject followed immediately.

In two test series (*e* and *f*, Table I) the tentacles of intact anemones which had settled on clean *Mytilus* shells (and therefore could be moved with little disturbance) were carefully brought into direct contact with tentacles of subjects. These were scored in the same manner as described above: if on moving the animals apart tentacles adhered, a positive was scored; if not, a negative.

Results

Preliminary tests using solitary and clonal *A. elegantissima* gave no evidence of tentacular adhesion. Hence no further experiments were carried out with solitary animals.

Table I

Combined data from tests for tentacular adhesion to prepared glass rods and to intact animals in two phenotypically distinct clones of Anthopleura elegantissima. *In each series the N equals 100 tests for adhesion of individual, randomly selected tentacles. Positive indicates adhesion, negative non-adhesion.*

Stimuli	Positive	Negative
a. Clean rod (control)	1	99
b. Rod plus saliva (control)	93	7
c. Rod plus clonemate mucus	2	98
d. Rod plus non-clonemate mucus	4	96
e. Intact clonemate	1	99
f. Intact non-clonemate	39	61

TABLE II

Combined data from tests for tentacular adhesion in two clones of Anthopleura elegantissima *upon contact with glass rods carrying the mucus of four Pacific Zoantharia. N = 100.*

Mucus from:	Postive	Negative
A. xanthogrammica	6	94
Metridium senile	96	4
Tealia lofotensis	98	2
Corynactis californica	92	8

Experiment 1 was designed to test the hypothesis that the tentacular nematocytes of clonal *A. elegantissima* animals respond differently to the mucus of clonemates and non-clonemates. One series of tests was conducted each day for six days. Four of these series (*a–d*, Table I) involved the glass rod technique; in two series, *e* and *f*, animals were brought into direct contact. Each series involved ten subject anemones (five from each of the two different clones). Tests of a single, randomly selected tentacle of each animal were followed by a two-minute rest period. This was repeated ten times, giving a total of 100 tests per series.

Experimental protocol and results, as seen in Table I, clearly indicate that no significant discharge takes place in response to isolated mucus from either clonemate or non-clonemate. Thus, the hypothesis is untenable. However, the last two series showed that direct contact with an intact non-clonemate effects significant discharge, when such contact with a clonemate does not.

Experiment 2 tested the hypothesis that the nematocytes of *A. elegantissima* respond differently to the mucus of its congeneric relative *A. xanthogrammica* than they do to the mucus of more distantly related Zoantharia. Table II supports the hypothesis and indicates that while contact with *A. xanthogrammica* mucus elicits little discharge, heavy discharge occurs upon contact with the mucus of three non-congeneric Zoantharia, *Metridium senile, Tealia lofotensis* and *Corynactis californica.*

Experiment 3 tested the hypothesis that mucus from *A. elegantissima* will give the same spectrum of responses when tested against the tentacles of the four Zoantharia used as mucus donors in Experiment 2. The data of Table III disprove the hypothesis, for mucus from *A. elegantissima* causes heavy discharge in the congeneric *A. xanthogrammica*, whereas (Table II), mucus from *A. xanthogrammica* has little effect on *A. elegantissima.* In addition, while mucus from *A. elegantissima* effects little discharge in *Metridium senile*, mucus from *M. senile* causes heavy discharge in *A. elegantissima* (Table II).

TABLE III

Adhesion of individual tentacles upon contact with a control glass rod and with a rod carrying the mucus of Anthopleura elegantissima *in four Pacific Zoantharia. N = 100.*

Zoantharian	Clean glass rod (% adhering)	Rod with *A.elegantissima* mucus (% adhering)
A. xanthogrammica	0	92
M. senile	3	11
T. lofotensis	0	87
C. californica	11	76

DISCUSSION

The present study indicates that the system of tentacular nematocyte discharge in *Anthopleura elegantissima* discerns no difference in the mucus of clonemate and non-clonemate. Nevertheless, factors in the mucus may be involved in the recognition response that results in the avoidance of non-clonemates (Francis, 1973b). In our experiments, almost invariably upon the application of clonemate or non-clonemate mucus to the tentacle of a subject animal, a retraction occurred, often of neighboring tentacles and occasionally of the whole tentacular crown. A clean glass rod elicited no such reaction. This avoidance response is identical to that observed after contact between intact non-clonemates and briefly after initial contact between clonemates that have been isolated for a time. However, the agent in the mucus that elicits these overt intraspecific recognition responses does not appear to affect the discharge system of the tentacular nematocytes.

One of the most interesting facts to emerge from this study is that the discharge system of *A. elegantissima* treats the mucus of the congeneric *A. xanthogrammica* as self, while the converse is not true: the *A. xanthogrammica* system treats the mucus of *A. elegantissima* as non-self. The mucus of *A. elegantissima* must contain substances which trigger discharge in non-congeneric forms as seen in Table II. In some way the discharge system of *A. elegantissima* must be "buffered" against the effector-substance(s) present in its own mucus and that of *A. xanthogrammica*.

Active and passive models can explain these results. The passive model assumes that if an agent is applied to a tentacle and no discharge occurs, then either no effector-substance is in the agent or if one is, it is simply ineffective in lowering the threshold of the specific discharge system being tested. According to this model the effector-substance in the mucus of *A. elegantissima* that lowers the threshold of discharge in other Zoantharia is simply without effect on the animal's own system.

The active model (Schlichter, 1976) assumes that Zoantharia mucus contains an inhibitory agent which "protects" the animal's own discharge system against the effector-substances present in it. Although this model assumes a high level of species-specificity, we find it more tenable than the passive one. It accords with the suggestion of Lentz and Barnett (1962) that in *Hydra* discharge-effector substances are enzyme substrates which react with strategically placed enzymes on the nematocyte or cnidocil to effect discharge. These workers found that various organic phosphates augment nematocyte discharge and that discharge to the phosphates is inhibited by known enzymatic inhibitors. Such a system provides opportunities for the diversity in specificity which has been shown to exist in many experiments to date.

A recent study by Lubbock (1980) is relevant. Lubbock compared the specificity of the acrorhagial system in *A. elegantissima* with that of the tentacular system. He showed that neither group of nematocytes responds to excised syngeneic (clonemate) tissue, but both respond to allogeneic (non-clonemate) tissue. Tentacular nematocytes respond to a broad range of tissues from diverse taxa, while acrorhagial ones are far more specific and discharge only to certain allogeneic and xenogeneic (non-conspecific) Zoantharia. Our results concur that isolated mucus fails to elicit discharge in allogeneic animals (non-clonemates) as well as syngeneic ones, but that direct contact with an allogeneic individual (either the intact animal or tissue excised from it) effects discharge.

As yet we have no real knowledge of how recognition is mediated at the nematocyte, or of the stimulus-to-discharge chain. Some workers (Bigger, 1980) have considered whether antigen-antibody phenomena are involved in discharge. Hildemann *et al.* (1979) pointed out that the minimum criteria for an immunologic phenomenon have not been established in a nematocyte discharge system. One criterion is "inducible memory or selectively altered reactivity on secondary contact." In a brief experiment Lubbock (1980), using acrorhagial nematocyte discharge as the criterion, was unable to show that repeated aggressions against one particular clone specifically enhanced the aggressive response to that clone.

Many questions about the nematocyte discharge system remain unanswered. Although spatially limited, the system is highly sophisticated and is affected by agents working at different points. These points are unknown. We still do not understand how the effects of mechanical and chemical stimulation are coupled. Moreover, we know little or nothing about changes in discharge level over time, though that these changes occur is well established (Davenport *et al.*, 1961; Ross and Sutton, 1964; MacFarlane and Shelton, 1975).

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